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Direct Incorporation of [^{11}C]CO_2 into asymmetric [^{11}C]carbonates

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Abstract

A novel carbon-11 radiolabelling methodology for the synthesis of the dialkylcarbonate functional group has been developed. The method uses cyclotron-produced short-lived [^{11}C]CO_2 (half-life 20.4 min) directly from the cyclotron target in a one-pot synthesis. Alcohol in the presence of base trapped [^{11}C]CO_2 efficiently forming an [^{11}C]alkylcarbonate intermediate that subsequently reacted with an alkylchloride producing the di-substituted [^{11}C]carbonate (34% radiochemical yield, determined by radio-HPLC) in 5 minutes from the end of [^{11}C]CO_2 cyclotron delivery.

Introduction

Positron emission tomography (PET) is an imaging technique able to detect and monitor specific target proteins *in vivo*.[1] The use of PET imaging has advanced in the last few decades to become a valuable tool in clinical diagnostics, medical research and drug discovery.[2] PET relies on the use of tracer amounts of imaging probes (radiotracers). The administration of radiotracers allows biochemical process to be imaged and quantified *in vivo* without manifestation of pharmacological or toxicological effects.[3]

Carbon-11 (^{11}C) is one of the most common radionuclides used for the synthesis of PET radiotracers. The short half-life of ^{11}C (20.4 min) makes it an attractive radionuclide as it enables the collection of a sufficient amount of PET data while keeping the subject radiation dose and exposure time to minimum. Furthermore it allows orthologus substitution with

carbon-12 in biologically active molecules with no alteration of the parent molecule's physico-chemical and pharmacological properties. Carbon-11 is commonly produced in the form of [^{11}C]carbon dioxide ([^{11}C]CO_2).[4] [^{11}C]CO_2 is usually converted into more reactive secondary precursors such as [^{11}C]methyl iodide ([^{11}C]CH_3I), [^{11}C]carbon monoxide ([^{11}C]CO), and [^{11}C]phosgene ([^{11}C]$COCl_2$).[5] As these multistep conversion processes are time consuming, the use of [^{11}C]CO_2 for directly radiolabelling functional groups is highly attractive.

[^{11}C]CO_2 is a weak electrophile with an affinity for electron-donating reagents such as amines and organometallics.[6] However, due to the thermodynamic and kinetic properties of [^{11}C]CO_2, it has high activation energy which requires the use of highly reactive reagents, temperatures, pressures, or the presence of a catalyst.[7] Nevertheless, the primary synthon, [^{11}C]CO_2, has been deployed successfully for the synthesis of ^{11}C-compounds that contain carbonyl groups such as [^{11}C]carbamates,[8] amide,[9] and [^{11}C]ureas.[10] However, the radiolabelling of the carbonyl group of carbonates from [^{11}C]CO_2 has not yet been established. To date, the synthesis of [^{11}C]carbonates has relied on the use of [^{11}C]$COCl_2$ which is produced from a multistep process starting from cyclotron-produced [^{11}C]CH_4, conversion to [^{11}C]CCl_4 and then to [^{11}C]$COCl_2$.[11] Although this ^{11}C-carbonate reaction is rapid and efficient, routine production of [^{11}C]$COCl_2$ requires a multistep syntheses and specialized equipment, thereby restricting its widespread use.[11]

As the carbonate functional group is found in prodrug compounds as well as being an intermediate in organic synthesis [12] we aimed to develop a simple and robust radiolabelling methodology that uses [^{11}C]CO_2 for the synthesis of [^{11}C]carbonates. Here we present a rapid, one-pot radiosynthetic strategy using [^{11}C]CO_2 directly from the cyclotron, avoiding the need for specialized equipment and multistep syntheses.

Materials and Methods

All purchased chemicals were used without further purification. Chemicals were purchased in highest available purity from Sigma-Aldrich and Alfa Aesar and used as received (> 99 % purity). All solvents were purchased as anhydrous in highest available purity (> 99.8 % purity) from Sigma-Aldrich.

[^{11}C]CO_2 was produced by a Siemens RDS112 cyclotron (St Thomas' Hospital, London, United Kingdom) via the ^{14}N(p,α)^{11}C nuclear reaction. Typical irradiation times for exploratory work was 1 minute,10 μA, bombardment typically yielding ca. 300 MBq [^{11}C]CO_2 at end of

cyclotron bombardment. Radiolabelling reactions were performed in a 1.5 mL screw top vial with a “V” internal shape. HPLC analysis was performed on an Agilent 2060 Infinity HPLC system with a variable wavelength detector (254 nm was used as default wavelength). An Agilent Eclipse XDB-C18 reverse-phase column (4.6 x 150 mm, 5 µm) was used at a flow rate of 1 mL/min and H_2O/MeOH (HPLC grade solvents with 0.1 % TFA) gradient elution (flow rate: 1 mL/min, 0-2 min: 5 % MeOH, 2-11 min: 5 to 95 % MeOH linear gradient, 11-13 min: 90 % MeOH, 13-14 min: 90 % to 5 % MeOH linear gradient, and 14-15 min: 5 % MeOH). The RCY was estimated by radio-HPLC and defined as the area under the ^{11}C-product peak expressed as a percentage of the total ^{11}C labelled peak areas observed in the chromatogram. Molar radioactivity was calculated from analytical HPLC sample of 25 µL. A calibration curve of known mass quantity versus HPLC peak area (254 nm) was used to calculate the mass concentration of the 25 µL radiolabelled compound. The identity of the radiolabelled compound peak was confirmed by HPLC co-injection of a nonradioactive reference compound and yielded a single peak.

Results and Discussion

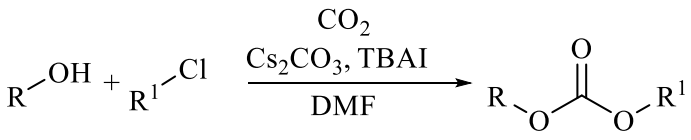
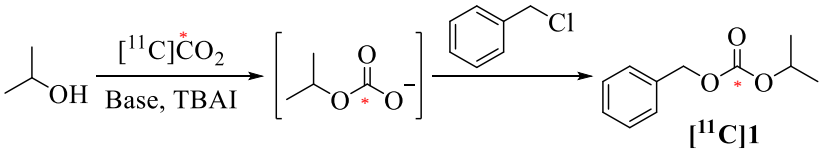


Figure 1. Method by Salvatore *et al. [7]* for the synthesis of carbonates using non-radioactive CO_2.

As a starting point we selected the method developed by Salvatore *et al.* [7] (**Figure 1**) for the synthesis of carbonates. The established method used non-radioactive CO_2, an alcohol derivative and benzylchloride (BzCl) in the presence of Cs_2CO_3, TBAI in DMF to produce the corresponding carbonate derivative efficiently. By substituting CO_2, with $[^{11}C]CO_2$ and applying the same reaction conditions, the synthesis of di-substituted $[^{11}C]$carbonates was investigated.

Table 1 Optimisation of [^{11}C]**1** synthesis.



[^{11}C]1

Entry[a]	Base	Trapping efficiency (%)	Temperature (°C)	Solvent	RCY (%)[b]
1	Cs_2CO_3	95.2	25	DMF	24
2	Cs_2SO_4	1.5	25	DMF	0
3	CsI	4.3	25	DMF	5
4	CsF	33.5	25	DMF	0
6	K_2CO_3	10	25	DMF	0
7	$CaCO_3$	0	25	DMF	0
8	Cs_2CO_3	20	25	CH_3CN	0
9	Cs_2CO_3	65	25	DMSO	0
10	Cs_2CO_3	>95%	65	DMF	33
11[c]	Cs_2CO_3	>95%	100	DMF	82, 74

[a] Reaction conditions: Isopropanol (22 μmol), Cs_2CO_3 (66 μmol), TBAI (66 μmol) and organohalide (66 μmol) in 500 μL DMF, 10 mins from end of delivery (EOD) (n=1). [b] The non-isolated radiochemical yield determined by radio-HPLC analysis of the crude product. [c] n=2

[^{11}C]CO_2 was trapped in isopropyl alcohol in the presence of Cs_2CO_3, forming an [^{11}C]isopropylcarbonate intermediate that subsequently reacted with BzCl to produce [^{11}C]benzyl isopropyl carbonate ([^{11}C]**1**) in a moderate radiochemical yield (RCY)[13] of 24% (Table 1, entry 1). Interestingly, almost all the cyclotron-produced [^{11}C]CO_2 was trapped within the reaction mixture at room temperature (> 95%); any unreacted radioactive [^{11}C]CO_2 was immobilized on an ascarite trap connected to the vial vent needle.[14]

In an attempt to increase the RCY, Cs_2CO_3 was replaced with Cs_2SO_4 (Table 1, entry 2). The trapping efficiency of $[^{11}C]CO_2$ dropped significantly from 95.2% to 1.5%. Since Cs_2CO_3 contributed towards the trapping of $[^{11}C]CO_2$ efficiently, we investigated whether the Cs^+ or the CO_3^{2-} ion was responsible for the high $[^{11}C]CO_2$ trapping efficiency. Of a number of caesium bases explored (Table 1, entries 3-5), CsI and CsF trapped only minute amounts of $[^{11}C]CO_2$ (4% and 34%, respectively), indicating that the basicity of the reaction mixture had a major effect on trapping efficiency. These results can be explained by the ability of a strong base to deprotonate the alcohol present in the reaction mixture enabling it to react with $[^{11}C]CO_2$ to form a ^{11}C radiolabelled intermediate. The importance of CO_3^{2-} was then explored by comparing Cs_2CO_3 with other carbonate bases (K_2CO_3 and $CaCO_3$, Table 1, entries 6 and 7). The trapping efficiencies were extremely low for both reagents. High trapping in the reaction mixture with Cs_2CO_3 is therefore most likely due to its superior solubility in organic solvents.

Table 2 Optimisation of $[^{11}C]$**1** synthesis using alternative bases.

Entry[a]	Base (eq)	TBAI (eq)	Temp (°C)	RCY (%)[b]
1[c]	DBU (3)	3	100	6
2 [c]	DBU (3)	-	100	0
3	NaH (1)	1	100	26
4[d]	NaH (1)	1	60	31±2
5 [c]	NaH (0.5)	1	60	18
6	NaH (2)	1	60	0
7[c]	NaH (0.5)	-	60	6
8	NaH (1)	3	60	7

[a] Isopropanol (1 equiv., 22 μmol), BzCl (3 equiv.), TBAI (1-3 equiv.) and base (1-3 equiv.) in 500 μL DMF reaction time 5 mins from EOD. [b] The non-isolated radiochemical yield determined by radio-HPLC analysis of the crude product. [c] Reaction time of 10 mins from EOD. [d] n=3

In a further attempt to increase the RCY of [^{11}C]**1**, a number of aprotic solvents were screened (CH_3CN and DMSO, Table 1, entries 8 and 9). However, these solvents did not produce [^{11}C]**1** and the trapping efficiency was poor (20% and 65%, respectively). Reaction dependency on temperature was subsequently examined. The RCY of [^{11}C]**1** improved from 24% to 33% by increasing the reaction temperature from 25 ^{o}C to 65 ^{o}C (Table 1, entry 10). Increasing the temperature to 100 ^{o}C promoted the product formation and resulted in the highest observed RCY (82%, Table 1, entry 11). This might be rationalised by an increase in Cs_2CO_3 solubility at higher temperatures. However, due the presence of Cs_2CO_3 as a reagent, low molar activities (A_m) were observed. The low A_m (2 GBq/μmol in this case) is likely due to release of non-radioactive CO_2 from Cs_2CO_3. CO_3^{2-} deprotonates the alcohol to form HCO_3^-, which at high temperature has the potential to decompose releasing non-radioactive CO_2 causing isotopic dilution and low A_m of the [^{11}C]CO_2. We therefore focused on improving A_m by substituting Cs_2CO_3 with an alternative base.

1,8-diazabicyclo[5.4.0]undecene (DBU) is a basic amine that has been shown to retain [^{11}C]CO_2 in organic solutions.[9] Replacing Cs_2CO_3 with DBU (Table 2, entry 1) resulted in [^{11}C]**1** formation, but with low RCY (6%). The low RCY could be due to DBU being unable to deprotonate isopropyl alcohol efficiently. We opted for a stronger base, NaH, which was able to deprotonate the isopropyl alcohol. Using a ratio of 1:1 NaH:isopropanol (equiv.) at 100 ^{o}C, [^{11}C]**1** was obtained with a RCY of 26% (Table 2, entry 3). Decreasing the temperature from 100 ^{o}C to 60 ^{o}C slightly improved the RCY (31%, Table 2, entry 4).[15] Decreasing the ratio of NaH:isopropanol (from 1:1 to 0.5:1) reduced the RCY further to 18% (Table 2, entries 5). Increasing the ratio NaH:isopropanol 2:1 did not produce the target product (Table 2, entry 6). Increasing the amount of TBAI to 3 equiv. or removing it completely also did not improve the RCY (Table 2, entries 7 and 8).

Conclusions

In conclusion, we have developed a radiolabelling methodology for the synthesis of [^{11}C]carbonates using [^{11}C]CO_2 directly from the cyclotron. The carbonate [^{11}C]**1** was synthesized by bubbling [^{11}C]CO_2 into a solution containing alkylchloride, alcohol and a base in DMF. The choice of the base was critical for maximising the RCY and A_m. The first protocol uses Cs_2CO_3 and produces the target ^{11}C radiolabelled product in a high RCY and low A_m. The second strategy, which uses NaH, produced [^{11}C]**1** in high A_m and moderate RCY. These

methodologies are a simple and practical alternative to ^{11}C-phosgene for the synthesis of ^{11}C-carbonates. ^{11}C-phosgene synthesis is technically challenging to implement and requires the use of specialist equipment. The developed strategies described here use readily available labware and converts [^{11}C]CO_2 directly to [^{11}C]carbonates in rapid synthesis times.

Data Availability

The necessary data used to support the findings of this study are included within the article. Any additional data that may be of interest to readers are available from the corresponding author upon request.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Funding Statement

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13. The RCY is the non-isolated radiochemical yield determined by radio-HPLC analysis of the crude product.
14. The trapping efficiency is the amount of radioactivity trapped in the reaction vial as a percentage of the overall radioactivity produced by the cyclotron.
15. [^{11}C]1 was produced with a molar activity (A_m) of 10 – 20 GBq/umol. This is because short cyclotron bombardments (1 minute) and low beam currents (5 – 10 μA) were used (0.3 GBq). In clinical productions at our facility, cyclotron bombardment times of 50 minutes and beam currents of 30 μA are

used to produce higher amounts of radioactivity (typically 60 GBq). It is therefore estimated that this would increase the A_m to > 50 GBq/μmol at end of synthesis.